Helicobacter pylori and Microsatellite instability in gastric tumors with hypermethylation of *E-cadherin* and *CDKN2A*

Adriana C. Ferrasi[1], Maria Inês. M. C. Pardini[1], Cristiane M. Thomazini[2], Marcia C. R. Pena[1], Silvia H.B. Rabenhorst[3], Maria A. M. Rodrigues[2], Nídia A. Pinheiro[1]

[1] Lab. de Biologia Molecular – Hemocentro-FM/UNESP-Botucatu

[2] Departamento de Patologia FM/UNESP-Botucatu/SP

[3] Departamento de Patologia e Medicina Legal/UFC-Fortaleza-CE

Gastric Cancer (GC) is one of the more common types of cancer presenting a high mortality frequency. Histologically it is divided into intestinal or diffuse types both of which are substantially diverse in epidemiology and pathogenesis. One of the more important etiological factors in GC is the infection by *Helicobacter pylori* (*HP*), a Class I carcinogen (WHO). The complex interactions among the different types of HP, inflammation and genetic features of host should promote a cascade of morphologic events and finally lead to CG of the intestinal type. Studies have demonstrated that many genes involved in the regulation of cellular cycle, tissues invasion, repair of DNA and apoptosis can be silenced by hypermethylation of gene promoters, essential in the progression of GC. The gene E-Cadherin (*CDH-1*) codifies a molecule of cellular adhesion, also considered a potential metastasis suppressor and invasion tumor. About 50% of CG of the diffuse type presents mutations in this gene, and other types of inactivation occur through hypermethylation. The gene *CDKN2A* codifies a protein that inhibits cyclin-dependent kinases (CDKs), which drive the progression of the cell cycle. The presence of the virus *Epstein Barr* is found in 2-16% of GC and seems to be related to hypermethylation of some genes, especially *CDKN2A*. The microsatellite instability (MSI) is observed in 20-30% of CG and the *locus* monomorphic BAT-26 is highly sensitive to MSI. Our study aimed to evaluate the presence of HP and

EBV in samples of gastric tumors and to analyze the frequency of MSI and the pattern of methylation on the genic promoters of *CDH-1* and *CDKN2A*. Ninety-nine samples of gastric tumors were used (intestinal and diffuse types, stage I to IV) and respective normal adjacent tissue. The presence of EBV and HP genotyping was detected by the Polymerase Chain Reaction (PCR) with specific primers. The frequency of MSI was verified by PCR with one of the primers marked by fluorescin 6-FAM and subsequent analysis in the sequencer ABI 3100 system. The methylation pattern in *CDH-1* and *CDKN2A* was determined by the MSP-PCR technique. The presence of HP was detected in 89 (97.8%) of the 91 analyzed samples and of those, 64% were *CagA*+ and 94.4% positive *VacAs1*. EBV was found in 73.6% of the samples of gastric tumors. The analysis of BAT-26 revealed 16.5% of the cases with positive MSI. Until the moment, thirty samples of CG and tissue normal adjacent were appraised for the methylation pattern in the genes *CDH-1* and *CDKN2A*. The methylation frequencies for *CDH-1* were 53.3%(16/30) and 76.6%(23/30) in the normal and tumor tissue, respectively and in *CDKN2A*, 33.3%(10/30) and 50%(15/30) in the normal and tumor tissue, respectively.

Key words: *Helicobacter pylori*, *Epstein Barr* Virus, microsatellite, instability, methylation, MSP-PCR, gastric cancer